REMARKS

Claims 1-5, 7, 9-12, 25-28, 48 and 51-54 are pending in the subject application. Applicants hereinabove have amended claim 25. Support for the amendment to claim 25 may be found, *inter alia*, in the specification as detailed in the following table:

Claim 25	Amendment	Support
step a)	indication that the human adipose tissue is obtained from a newborn to a eight year old child	page 6, line 21; page 7, lines 10-12
step b)	editorial changes in order to clarify the wording of step b)	page 6, lines 22-23; page 8, lines 1-4;
step c)	indication that the culture is carried out for 12 hours	page 6, line 24; page 8, liens 18-19;
step d)	editorial changes in order to clarify the wording of step d)	page 6, lines 11-13 and 25-27; page 9, lines 5-10;
step e)	indication that the cells are cultured for 50 to 80 population doublings and diluted by a maximum of two or three at each transfer	page 6, lines 14-16 and 28-29; page 8, lines 18-19; page 9, line 31; page 10, lines 11-13;
step f)	indication that step f) is optional and editorial changes in order to clarify its wording	page 6, lines 16-17 and 30-31;
step g)	added step reciting the recovery of the stem cells	page 12, lines 10-11

Upon entry of this Amendment, claims 1-5, 7, 9-12, 25-28, 48, and 51-54, as amended, will be pending and under examination.

Claim Rejection Under 35 U.S.C. §112

Applicants note that the Examiner has withdrawn the rejection previously made under 35 U.S.C. §112.

Claim Rejections 35 USC §103

The Examiner has rejected claims 1-12, 25-28, 48, 51-54 as filed on September 29, 2008 under 35 U.S.C. 103(a) as allegedly obvious over Katz et al., in view of Akanbi et al., Hedrick et al. and Haynesworth et al.

In particular, the Examiner considers that the skilled person would find guidance in Akanbi et al. to obtain cells from younger patients, that Hedrick et al. teach that non adherent cells are removed after 12 hours, and that the combination of the teaching of Katz et al., Akanbi et al., Hedrick et al. and Haynesworth et al., would lead to the claimed cells.

In response, Applicants submit that the subject-matter of the pending claims as amended hereinabove is not obvious over the cited references for the reasons detailed hereafter.

1. The specific process used by the inventors allows one to obtain a highly homogeneous stem cell population with novel properties

The method recited in amended claim 25, which was used by the inventors to isolate the claimed stem cells, involves performing a

number of steps which allows one to obtain a highly homogeneous population of stem cells with novel properties.

The effect of each of these steps is detailed in the following table :

Method step	**Technical effect**
step (a) : enzymatically digesting a sample of human adipose tissue obtained from a one-month old to 8 year old child;	This step allows complete dissociation of the tissue (page 7, lines 7-8) and helps to minimize potential aging effects on stem cell properties
step (b) : recovering from the digested sample obtained in step a) a cell fraction that is free of adipocytes, said cell fraction containing all of the cell types present in said sample with the exception of adipocytes;	This step allows one to obtain an adipocyte free cell fraction which retains all the other cell types (page 8, lines 1-4). Removing only the adipocytes is important because at this stage the proportion of stem is very low. Thus, removing other cell types would increase the risk of removing the stem cells.

Method step	**Technical effect**
step (c) : carrying out in vitro culture of the cell fraction obtained in step (b) for 12 hours; step (d) : selecting from the *in vitro* cell culture of step c) the cells which have adhered 12 hours after starting the culture, to obtain a cell sub-population termed "CA" ;	These steps allow the selection of a cell population comprising a higher proportion of true stem cells because stem cells adhere to plastic in 12 hours or less (see page 9, liens 26-28). These steps are essential for obtaining a cell population in which the true stem cells are not only present, but are not too diluted so as to thereby minimize the risk of "losing" the stem cells when transferring and diluting the cell populations.
step (e) : culturing the "CA" sub population of cells *in vitro* for 50 to 80 population doublings and diluting the cells a maximum of two or three fold at each transfer until a quiescent population of cells is obtained;	Diluting the cells by a maximum of two or three fold at each transfer is important since a greater dilution would increase the risk of losing the stem cells in certain culture dishes (see page 10, lines 14-17 of the specification). Further, culturing the cells until they enter into quiescence allows one to increase the concentration of true stem cells. Indeed, other cell types progressively die off while the true stem cells continue cell division (see page 10, lines 2-5). Further performing of 50 to 80 population doublings allows one to obtain cells whose HLA Class I phenotype is negative. Indeed, during the first population doublings the HLA Class I phenotype of the "CA" cells is low but significant, and after 50 to 80

Method step	**Technical effect**
	population doublings it disappears (see page 14, lines 17-21). Performing of the culture step e) is thus essential for obtaining stem cells which are HLA Class I negative. Moreover, as other cell types do not enter into quiescence, this step allows one to select a cell population consisting only of true stem cells. These features are essential for the obtaining a **homogeneous** population of true stem cells which have an HLA Class I negative phenotype.
step (f) : optionally, inducing proliferation of the quiescent population of cells obtained in step e);	This optional step allows, once an homogeneous stem cell population is obtained, one to increase the number of stem cells.
step (g) : recovering the cells obtained in step e) or f), so as to recover stem cells.	This step allows the recovery of a highly homogenous population of true stem cells.

This method was conceived by the inventors as one which would allow the selection of a stem cell population comprising stem cells having the novel phenotype of the claimed cells and no other cell types.

Indeed, performing of steps a) to g) allows one to obtain a novel stem cell population which is highly homogeneous and HLA Class I negative, and thus is highly suitable for therapeutic and cosmetic applications.

In particular, step (e) allows selection of true stem cells and an

increase in their number because most of the other cell types present after step (d) (e.g. precursor cells...) die before 50 to 80 population doublings and those which do not die are not capable of entering into quiescence. After 50 to 80 population doublings, the remaining cells which are not stem cells enter into senescence and die leaving only the true stem cells in the living cell population. The resulting claimed stem cells are capable of undergoing at least 130 population doublings, for example, over 200 population doublings, and of entering into quiescence.

Further, a number of population doublings needs to take place before an HLA Class I negative phenotype is detected. Step (e) thus also allows the obtaining of a population of stem cells which are HLA Class I negative.

2. Surprising effects

As indicated above, the inventors have discovered that the HLA Class I phenotype of the CA cell population obtained after performing steps a) to d) is positive but becomes negative during the course of step e). This change of phenotype is neither taught nor suggested no predictable from the cited art. The effect of the number of population doublings on the HLA Class I phenotype of the CA population was thus totally unexpected.

The inventors have thus identified not only a means to purify stem cells but also a method to ensure obtaining a cell population which has a specific phenotype, in particular, an HLA Class I negative phenotype.

As there was no reason to expect this change would occur, the skilled person had thus no reason to carry out step e).

3. Advantages of the claimed cells

It is stressed that the **homogeneity** of a claimed stem cell population and the fact it contains a **high number of cells** are two essential pre-requisites to using stem cells in therapeutic and cosmetic applications. An **HLA Class I negative phenotype** is also an important pre-requisite to envisaging allo-transplantation of these cells in a human being with no risk of rejection (see page 14, lines 11-12 of the specification and section 12.2.2 of the examples).

The method developed by the inventors, and in particular step e) thus allows one to obtain a novel stem cell population which has the advantage of being highly suitable for use in therapy and cosmetic applications.

4. The method for obtaining the claimed cells cannot be derived from the cited art

The following chart displays in the first column the method steps recited in claim 25. In the other columns, the presence or absence of these steps in the cited documents is indicated as "yes" or "no" respectively.

Method step	**Katz et al.**	**Akanbi et al.**	**Hedrick et al.**	**TOTAL**
(a) enzymatically digesting a sample of human adipose tissue obtained from a one-month old to 8 year old child;	No (elective surgery, i.e. performed on adults)	No (one-day old pig cells)	No (elective surgery, i.e. performed on adults)	**No**
(b) recovering from the	Yes (centrifugation,	No, as filtration step removes	No, as filtration step removes	**Yes**

digested sample obtained in step a) a cell fraction that is free of adipocytes, said cell fraction containing all of the cell types present in said sample with the exception of adipocytes;	page 17)	other cell types (page 8 of specification)	other cell types (page 8 of specification)	
(c) carrying out in vitro culture of the cell fraction obtained in step (b) for 12 hours;	No (no mention of length of time for which cells are cultured)	No (it is specified that cells are cultured for 24h)	No (Hedrick et al. does not disclose culture of a cell fraction corresponding to that obtained in step (b))	**No**
(d) selecting from the *in vitro* cell culture of step c) the cells which have adhered 12 hours after starting the culture, to obtain a cell sub-population termed "CA" ;	No	No, as adherent cells are removed after 24 hours	No (Hedrick et al. does not disclose culture of a cell fraction corresponding to that obtained in step (b))	**No**
(e)	No	No	No	**No**

culturing the "CA" sub population of cells *in vitro* for 50 to 80 population doublings and diluting the cells a maximum of two or three fold at each transfer until a quiescent population of cells is obtained;	(cells cultured for 10 to 20 passages, no mention of quiescence)	(no mention of quiescence)	(no mention of quiescence)	
step (f) optionally, inducing proliferation of the quiescent population of cells obtained in step e);	No (no mention of quiescence)	No (no mention of quiescence)	No (no mention of quiescence)	**No**
(g) : recovering the cells obtained in step e) or f), so as to thus recover stem cells.	No	No	No	**No**

As clearly shown above, four of the steps recited in claim 25 and performed by the inventors to obtain the claimed stem cells, i.e. steps a), e), f) and g), are <u>not</u> disclosed at all, and steps c) and d) not clearly disclosed in the cited references.

As indicated in section 3-1 above, performing **all** the steps of this method (with the exception of optional step (f)) is essential to obtain the claimed stem cells.

In particular, as indicated above, performing of step (e) allows one to obtain true stem cells which are HLA Class I negative, as well as selection and an increase in the number of such cells.

Indeed, step (e) consists in cultivating the "CA" cell population *in vitro* for 50 to 80 population doublings and diluting the cells a maximum of two or three fold at each transfer until a quiescent population of cells is obtained.

As indicated in the above table, step e) is essential for the obtaining of a homogeneous population of true stem cells which are HLA Class I negative in so far as :

- diluting the cells by a maximum of two or three at each transfer is important in order not to lose the stem cells in certain culture dishes;
- the CA cell population needs to undergo a number of population doublings before an HLA Class I negative phenotype is detected (page 14, lines 17-21 of the specification);
- culturing the cells until they enter into quiescence allows one to increase the number of stem cells while at the same time decreasing the number of other cell types including other multipotent cell types whose longevity in terms of population doublings is more limited, i.e. to increase the concentration of true stem cells ;
- the entering into quiescence step allows one to select a cell population consisting of true stem cells as the other cell types enter into senescence at this stage.

Step e) in particular is thus essential for the obtaining a homogeneous cell population comprising a high number of true stem cells which are HLA Class I negative.

In particular, it is indicated at page 14, lines 17-21 of the specification that before performing this step, the CA cell population has an HLA Class I positive phenotype.

Performing a method which does not comprise all the steps recited in claim 25, and in particular step e), thus does not allow to obtain the claimed cells.

As performing most of the method steps recited in claim 25 is **not** disclosed in the cited references, the skilled person would thus not be driven to perform **all of** these steps, and thus would not obtain the desired and claimed stem cells.

None of the cited references suggests that stem cells having the claimed properties exist in adipose tissue. Further, none of the cited references discloses a means to ensure that the stem cell population is HLA Class I negative and homogeneous. None of the cited references even suggests exploiting the long life span of the claimed cells whether to eliminate other cell types or to obtain a cell population which has a HLA Class I negative phenotype. Further, **none of these documents addresses the importance of cultivating the cells until they enter into quiescence**. In fact, none of these references addresses the problem of obtaining a stem cell population which is HLA Class I negative and homogeneous. The skilled person would thus have found no incentive in the cited references to develop a method to obtain such cells.

Thus, even when taken in combination, the cited references would not have driven the skilled person to perform the method recited in claim

25. As performing this method is necessary to obtain the claimed stem cells, the skilled person would not have obtained them.

Thus, neither the claimed cells nor the method for obtaining them are obvious over the cited references.

Step a) is not obvious over Katz et al. and Akanbi et al.

Contrary to the Examiner's assertion Akanbi et al. actually teaches away from using adipose tissue from a younger subject.

Akanbi et al. teaches that adipocyte precursor cells obtained from adipose tissue of younger pigs divide faster than those obtained from older pigs.

However, adipocyte precursor cells are not the cell type which the skilled person is seeking to isolate according to the subject invention. Precursor cells are indeed different from stem cells. In particular their potential for differentiation is much more limited because they are already committed to the adipocyte lineage and they divide faster.

Further, with regard to the objective of the method disclosed and claimed in the subject application, that is, the isolation and purification of a highly homogeneous stem cell population, not only are precursor cells not the cell type which the skilled person is seeking to isolate, but their presence in the cell population obtained is considered as the presence of a contaminant (see page 10, lines 11-15 of the specification).

The skilled person seeking to obtain a highly homogenous stem cell population will thus be driven to select a tissue in which precursor cells are present in a lower number and/or in which they proliferate at a slower rate.

Akanbi et al. teaches that adipocyte precursor cells proliferate faster when obtained from a younger subject. Thus, should the skilled person combine the teaching Akanbi et al. with that of Katz et al., he would actually be driven to select adipose tissue from an **older** subject in order to obtain a cell population in which the number of precursor cells is lower.

Consequently, Akanbi et al. does teach away from using adipose tissue from a younger subject.

Using adipose tissue from a young child, as recited in the claims, is therefore not obvious over the teaching of Katz et al. and Akanbi et al.

Conclusion

In conclusion, Applicants submit that

- the method recited in claim 25 was neither taught nor suggested nor obvious from the cited art. It is emphasized that the Examiner has not provided evidence to the contrary;
- the combination of the teaching of Katz et al. and Akanbi et al. would not lead the skilled person to use adipose tissue from a young child;
- the combination of the teaching of Katz et al., Hedrick et al. and Akanbi et al. would not lead the skilled person to perform the method developed by the inventors, and thus would not lead to obtaining the claimed cells;
- the finding that the HLA Class I phenotype of the CA cell population could be modified by implementing step e) is unexpected;
- the claimed cells, which have been acknowledged as novel by the Examiner, are highly suitable for use in therapy and cosmetics.

In particular, performing the method steps a) and e) which are essential for the obtaining the claimed cells, is neither taught nor suggested nor obvious from the cited art.

The skilled person would thus have found no guidance in the prior art to obtain the claimed stem cells, i.e. stem cells highly suitable for use in therapy.

Based on the amendment to claim 25 and the preceding remarks, applicants maintain that no combination of Katz et al., Akanbi et al., Heidreick et al., and Haynesworth et al. renders applicants' claimed invention obvious.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw this ground of rejection under 35 U.S.C. §103.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed $1,110.00 fee for a three-month extension of time and $810.00 fee for filing an RCE, is deemed necessary in connection with the filing of this Amendment and RCE. Accordingly, a check in the amount of $1,920.00 is enclosed. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Attorney for Applicants
Cooper & Dunham LLP
30 Rockefeller Plaza
20th Floor
New York, NY 10112
(212) 278-0400

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